PATENT COOPERATION TREATY

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B0131WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/08701	International filing date *(day/month/year)* 06.08.2003	Priority date *(day/month/year)* 08.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/24		
Applicant CYTHERIS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.02.2004	22.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Sirim, P Telephone No. +49 89 2399-7732

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/08701

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-65 as originally filed

Sequence listings part of the description, Pages

1-18 as originally filed

Claims, Numbers

1-55 received on 17.05.2004 with letter of 07.05.2004

Drawings, Sheets

1/18-18/18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-30, 37, 44-53
	No:	Claims	1-7, 31-36, 38-43, 54,55
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-55
Industrial applicability (IA)	Yes:	Claims	1-55
	No:	Claims	

2. Citations and explanations

see separate sheet

1. Documents

The following documents (D) have been taken into consideration; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-0 314 415 (IMMUNEX CORP) 3 May 1989 (1989-05-03)

D2: WO 00 17362 A (SCHERING CORP) 30 March 2000 (2000-03-30)

D3: WO 96 04306 A (SCHERING CORP) 15 February 1996 (1996-02-15)

D4: WO 99 03887 A (BOLDER BIOTECHNOLOGY INC; COX GEORGE N III (US)) 28 January 1999 (1999-01-28)

D5: US-A-5 459 058 (LEDER PHILIP ET AL) 17 October 1995 (1995-10-17)

D6: WO 01 75140 A (UNIV CONNECTICUT) 11 October 2001 (2001-10-11)

D7: SRINIVASAN S ET AL: 'A model of IL-7 and extra-cellular domains of its receptor complex using distance geometry and structure-function data.' PROTEIN ENGINEERING, vol. 6, no. SUPPL., 1993, page 107 XP009003649, Winter Symposium on Advances in Gene Technology: Protein Engineering and Beyond; Miami, Florida, USA; 1993 ISSN: 0269-2139

D8: KROEMER ROMANO T ET AL: 'Prediction of the three-dimensional structure of human interleukin-7 by homology modeling.' PROTEIN ENGINEERING, vol. 9, no. 6, 1996, pages 493-498, XP002226699 ISSN: 0269-2139

D9: GOODWIN RG ET AL: 'Human interleukin 7: molecular cloning and growth factor activity on human and murine B-lineage cells' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, XP002077974 ISSN: 0027-8424

D10: COSENZA LARRY ET AL: 'Disulfide bond assignment in human interleukin-7 by matrix-assisted laser desorption/ionization mass spectroscopy and site-directed cysteine to serine mutational analysis.' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 52, 26 December 1997 (1997-12-26), pages 32995-33000, XP002226701

ISSN: 0021-9258

D11: COSENZA LARRY ET AL: 'Comparative model building of interleukin-7 using interleukin-4 as a template: A structural hypothesis that displays atypical surface chemistry in helix D important for receptor activation.' PROTEIN SCIENCE, vol. 9, no. 5, May 2000 (2000-05), pages 916-926, XP002226702 ISSN: 0961-8368

2. **Subject-matter of the invention**
The present application relates to the preparation and use of a specific IL-7 conformer which is considered as the native configuration of the protein. Said IL-7 conformer comprises three disulfide crosslinks formed by the (1) 1st and 4th cysteine, (2) 2nd and 5th cysteine and (3) 3rd and 6th cysteine of the protein.

3. **Novelty (Art. 33(2) PCT) and Inventive step (Art. 33(3) PCT)**
3.1. The subject-matter of the present claims 1 to 7, 31-36, 38-43 and 45-55 is not novel in the sense of Art. 33(2) PCT, since the isolated protein, its DNA and amino acid sequences, the existence of three intramolecular disulfide bonds and the therapeutic use of its immune stimulatory function have been disclosed in any of the documents D1 to D12.

The sole assignment of the positions of the cysteine bonds of IL-7 does not render a cloned protein novel, since the 3D structure of a protein is an intrinsic feature resulting from its primary structure.

Furthermore, based on computational methods and homology studies either of the documents D7 (figure) or D8 (page 497, §2) have proposed **the identical disulfide pattern** for IL-7 as the present application. Further, in D7 modeling studies showed that the receptor binding which is required for the biological function of IL-7 depends of this disulfide pattern and that improperly folded analogues of said molecule show altered receptor binding (D7: results/discussion). The authors of D8 consider this IL-7 form as the "unique" form (see abstract) which "unambiguously" results from the topology of the six cysteines within the molecule (page 497, §2).

Thus, the prior art is aware of the correct 3D structure of IL-7 including the exact positions of the 3 disulfide bonds required to obtain a properly folded protein capable of binding to its receptor.

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The eukaryotic expression of the IL-7 as already disclosed in either of D1 (example 7), D2 (pages 51-56), D5 (columns 3-5) and D6 (example 7) will automatically result in a properly folded protein.

The method used in the present application for the bacterial expression of IL-7, comprising a first step of denaturation of the protein followed by a renaturation step resulting in a proper folded protein is just a standard method routinely followed by persons skilled in the art for the purification of proteins comprising intramolecular disulfide bonds.

Consequently, the subject-matter of the present claims 1 to 55 lacks an inventive step in the sense of Art. 33(3) PCT.